pennsylvania
DEPARTMENT OF ENVIRONMENTAL PROTECTION

BUREAU OF POINT AND NON-POINT SOURCE MANAGEMENT

RECREATIONAL USE ASSESSMENT METHODOLOGY

BACTERIOLOGICAL SAMPLING PROTOCOL

OCTOBER 2015

Applicability

This method applies to all surface waters of the Commonwealth of Pennsylvania, including streams, lakes, and reservoirs. The methods described below shall be used to assess recreational use attainment of surface waters. Use attainment decisions may be derived from other datasets on a case-by-case basis.

Sampling Frequency and Duration

Bacteriological sampling for determining water contact recreational use attainment should be conducted during the swimming season (May 1st through September 30th). . **A sampling group consists of 5 bacteriological samples collected on different days during a 30-day period and spanning a minimum of 14 days.** For bacteriological monitoring, the sampling period should capture the time frame when the public is likely to be engaging in primary water contact recreational activities. Sampling should not take place during those times when physical conditions such as stream discharge render primary contact recreation hazardous to the public. Typically, elevated fecal indicator levels are observed during periods of high discharge. However, people are unlikely to be participating in primary water contact activities during those conditions. Another consideration is that collecting samples during high flow conditions can be dangerous to the collector. Therefore PADEP recommends that monitoring bacteriological parameters for the purpose of Recreational Use Assessment occur during average or low flow conditions when primary contact recreation is likely to occur in a particular stream. This can be determined by using best professional judgment of average flow conditions for a particular stream. A general guidance is that sampling should not take place immediately following 0.25 inch of rain or more.

Sampling Approach

Targeted Water Contact Recreational Use Sampling Sampling efforts will focus on unassessed waters and waters that are heavily used for recreational activities by the public or those waters that support public beaches. Sampling locations will be chosen to characterize specific land use patterns or potential sources of impairment in a watershed. Impairment sources include municipal point sources, combined sewer overflows, and agricultural sources relating to manure application, livestock grazing, and animal feeding. Other targeted sites may be placed in areas of important recreational activities or where land use practices may contribute to potential bacteriological problems. Additionally, during the swimming season, the Department of Health (DOH) and Department of Conservation and Natural Resources (DCNR) collect weekly samples for *E.coli* at public beaches for monitoring purposes. *See* 28 Pa. Code Chapter 18, §18.30. Closure notices when violations of criteria occur at public beaches are also issued by DOH. In cooperation with DEP, DOH and DCNR provide a list of closures that DEP will utilize to focus future fecal coliform assessment sampling in areas where the closure lists indicate a possible recreational impairment.

Probabilistic Sampling

A probabilistic sampling design may be used, but in most instances a targeted design is employed. The purpose of the probabilistic sampling design is to determine the magnitude of the bacteriological problem over a large scale rather than at the small waterbody size. The probabilistic sampling design will require sampling at randomly selected sites in a watershed. The number of sites to be sampled in a watershed is based on the desired target precisions and estimated confidence levels.

The site selection process may be weighted on characteristics like Strahler stream order and land use. The actual site selection process is done using a grid system overlaid on a GIS map of all streams in the sample population, or all streams that could potentially be monitored for bacteriological analysis. During the grid process, the sample site locations are chosen at random. Alternatively, an R-script program developed by U.S. EPA Aquatic Resource Monitoring Western Ecology Division, Corvallis OR can be used to select random segments in a watershed based on reverse hierarchal ordering (two-stage Generalized Random Tessellation design).

Site Selection

Streams. Two sampling sites at a minimum (each with two completed sampling groups of five samples) are normally required to delineate an impaired stream segment but one station located at the mouth of a small tributary may be sufficient. Bacteriological data collection locations should be selected so that segments are properly bracketed by at least two sites, one upstream and one downstream from tributaries, point source discharges, and major changes in land use on a local level. In some cases more than two sites will be needed to assess a stream segment. If resources permit, it is desirable that multiple sites covering an entire watershed are sampled on the same set of days to compare daily bacterial levels spatially in a watershed while limiting temporal variance.

Lakes. Samples from lakes with beaches or designated swimming areas are collected from the left perimeter, right perimeter, and center of the swimming area. If the beach or swimming area is less than 100 feet in length then only two samples are collected, each one a third of the distance from either end of the perimeter. If there is more than one beach or designated swimming area on a lake then each one must be treated individually and sampled accordingly. Lakes one square mile or less that have no designated beach or swimming area require one sampling site at the outfall with two complete sampling groups of five samples. Lakes having a surface area greater than one square mile (640 acres) with no beaches require the use of best professional judgment to determine the location and number of sampling sites. The number and distribution of docks, boating areas, residences, cabins, and access areas should be considered in locating sample sites. Large lakes with beaches and heavy water ski and jet boat use may require additional sampling in non-beach areas.

Equipment

Bacteriological samples shall be collected using bottles that have been pre-sterilized and contain the proper amount of de-chlorinating agent (preservative). DEP supported sampling activities use 125-mL, screw-capped, polypropylene bottles with sodium thiosulfate added to neutralize the effects of residual chlorine.

Sample Collection Methodology

Ambient Surface Waters.
Streams: Sample in the main current, in the area of greatest flow (mid-channel, mid-depth). Do not sample at the water's edge and avoid stagnant water.

Lakes: Samples collected from the shoreline should be collected at a depth of one meter or knee depth at a minimum. Samples from a boat are collected with the anchor up (so as not to disturb the sediment), motor off, drifting toward or along shore, and water depth must be at least one meter.

a. Prior to sample collection, label the outside of the bottle with an adhesive label on the bottle or write the sample information directly on the bottle in permanent marker and cover with clear package tape.
b. Disposable gloves (elbow length preferred) are required for sample collection to ensure the safety of the sampler and reduce the potential of sample contamination.
c. After wading or boating to the desired sampling location, pause to allow disturbed sediment to settle. Do not collect water that is clouded by the sediment you disturb.
d. In flowing water, stand facing upstream.
e. Remove the cap from a 125-mL BacT bottle. Avoid touching the inside of the bottle, the threads at the top of the bottle, or the inside of the cap. If you accidentally touch any of these surfaces, a new bottle must be used.
f. DO NOT RINSE THE BOTTLE!
g. Sample water from a depth of 8 to 12 inches beneath the surface. If the water is less than 16 inches deep, collect the sample at mid-depth. If the water is less than 4 inches deep, find a new place to sample where the water is deeper.
h. Grasp the uncapped bottle near its base with the opening pointing downward and plunge it below the water surface. Turn the submerged bottle into the current and away from you. In slower current or lakes, push the bottle underneath the surface and away from you as if dipping water in an upstream direction.
i. DO NOT FILL THE BOTTLE COMPLETELY. Allow one-inch of air space. The sample is shaken at the laboratory prior to analysis and the air space makes this easier.

j. Recap the bottle carefully, making sure not to touch the inside of the bottle, the threaded top, or the inside of the lid and tighten.
k. Place each bottle in a cooler with ice. Place all laboratory forms in a plastic bag and place them inside the cooler on top of the ice.
l. Deliver samples to the laboratory within 6 hours of collection

Quality Control

Each collector shall submit one blank and one duplicate sample for every 10 samples submitted for analysis. The blank sample will be used to test the laboratory's accuracy, while the duplicate sample will be used to test the laboratory's precision.

1. *To complete a blank sample*: Fill a sample bottle with sterile (autoclaved) water from DEP's laboratory. Label the bottle consistent with regular water samples, **never indicate on the bottle that the sample is a blank**. Record the blank sample information on the same laboratory form as used for the regular water sample completed concurrently and do not indicate the sample is a blank.
2. *To complete a duplicate sample*: Take a second sample bottle to the monitoring site. After filling the first bottle (the test sample), immediately fill a second bottle (the duplicate sample) from the same location using the same technique. Label the duplicate sample as you would the test sample, but **do not indicate that the sample is a duplicate**. Record the duplicate sample information on the same laboratory form used for the regular water sample completed concurrently and do not indicate the sample is a duplicate.

Laboratory Results

Water Contact Recreation samples. Fecal coliform bacteria are reported by the laboratory as the number of colony forming units per 100 milliliters (cfu/100 mL). Analysis must be conducted by DEP-certified labs typically following Standard method 9222D (SIS code 31616) and must include recommended quality control analyses.[1] Other certified fecal coliform enumeration methods may also be considered by the Department.

Coastal Beach Samples. Coastal Beach samples are analyzed for *E.coli* bacteria and reported as the number of colony forming units per 100 milliliters (CFUs/100 mL). Analysis must be conducted by DEP-certified labs typically following EPA Method 1603 (SIS Code MMTECMF).[1] Other certified *E. coli* methods may also be considered by the Department.

Applicable Criteria

All waters of the Commonwealth with the exception of Lake Erie Coastal Beaches and waters specified with exceptions to the criteria in §93.9 a-z of 25 Pa. Code Chapter 93 are evaluated for water contact recreation use attainment according to the criteria for fecal coliform bacteria in §93.7 of Chapter 93 which specifies that during the swimming

season (May 1- September 30), the maximum fecal coliform level shall be a geometric mean of 200 cfu/100 mL based on a minimum of 5 samples collected in a 30-day period. In addition, no more than 10% of samples collected in a 30-day period shall exceed 400 cfu/100 mL.

Coastal Beach samples are evaluated for water contact recreational use attainment according to the E.coli standard referenced in the 28 Pa. Code §18.28 (b) (2) and (3) that specifies that a bathing beach will be considered contaminated for bathing purposes when either a 30-day geometric mean in all water samples collected exceeds 126 cfu per 100 mL or a sample exceeds 235 cfu/100 mL.

Data Processing

Water Contact Recreation and Coastal Beach Samples. A geometric mean is calculated for each sampling group (5 samples collected on different days in a 30-day period) at a site. To calculate a geometric mean of 5 samples, first compute the natural logarithm (ln) of each sample result and then calculate the average of the logarithm values. Finally, convert this product back to a normal value by computing the antilog of the product. The following examples of two sites on a waterbody illustrate how this is done:

Example Site 1

Sample 1:	130 cfu/100 mL	ln (130) = 4.868
Sample 2:	380 cfu/100 mL	ln (380) = 5.940
Sample 3:	240 cfu/100 mL	ln (240) = 5.481
Sample 4:	100 cfu/100 mL	ln (100) = 4.605
Sample 5:	180 cfu/100 mL	ln (180) = 5.193
		Average of logs = 5.217
		Antilog of average = 184.4

Example Site 2

Sample 1:	120 cfu/100 mL	ln (120) = 4.787
Sample 2:	390 cfu/100 mL	ln (390) = 5.966
Sample 3:	220 cfu/100 mL	ln (220) = 5.394
Sample 4:	130 cfu/100 mL	ln (130) = 4.868
Sample 5:	180 cfu/100 mL	ln (180) = 5.193
		Average of logs = 5.242
		Antilog of average = 189.0

[1] Laboratory certification by other nationally recognized certification organizations is also permissible.

Now compare the values 184.4 and 189.0 to the swimming season criterion for fecal coliforms (200 cfu/100 mL). A violation does not exist at the two example sites since neither value exceeds the criterion. We would conclude that the two example locations are attaining for recreational use.

PA DEP will assess waters as impaired for Recreational Use if one violation of the 30-day geometric mean fecal coliform criterion (or 30-day geometric mean PADOH E.coli criterion in the case of Coastal beaches) during the bathing season occurs. Samples may be collected throughout the bathing season at a site but a violation occurring during any 30-day period with a 5 day sample size and all 5 of the samples spaced more than 14 days apart will result in an impaired assessment status. The criteria also indicates that, no more than 10% of the grab samples collected from waters attaining recreational use exceed 400cfu/100 mL. The 10% exceedance will apply to assessment decisions on a case-by-case basis and only when there is enough data to support an impairment decision. Generally, the geometric mean will be used to assess waterbodies as that is more relevant to determining the long term water quality of a stream. Incomplete data sets that document consistently low bacteria levels are considered evidence that the waterbody is attaining the water contact recreational use if no likely sources of fecal coliform bacteria are present in the watershed.